Patent
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Sydney David FINKELSTEIN et al.	Group Art Unit: 1634
Application No.: 10/008,278	Examiner: Jeanine Anne Goldberg
Filed: November 5, 2001	Confirmation No.: 2727
For: TOPOGRAPHIC GENOTYPING	

DECLARATION OF SYDNEY DAVID FINKELSTEIN PURSUANT 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Sydney David Finkelstein, declare:

(1) That I am a named inventor on the instant application.

(2) That I am a United States citizen residing at 311 Marberry Drive, Pittsburgh, PA 15215.

(3) That I have been awarded a degree of M.D. from McGill University in Montreal, Canada, in 1977. I am Board certified in anatomic pathology (1984) and neuropathology (1984). I was a professor of pathology at the University of Pittsburgh from 2003 to 2004; I was an associate professor of pathology from 1993 to 2003 at the University of Pittsburgh. During 1993 to 2004 at the University of Pittsburgh, I was the Director of Molecular Anatomic Pathology and the Director of Gastrointestinal Pathology.

(4) That I am currently employed by RedPath Integrated Pathology, Inc. as Co-Founder and Chief Scientific Officer. I am also an adjunct Professor of Pathology at Drexel University.

(5) I have read and understood the Office Action mailed November 23, 2004. I have read and understood the references cited in the Office Action as applied in the rejections. The references are listed below:

(A) **Ben-Ezra** et al., "Effect of Fixation on the Amplification of Nucleic Acids from Paraffin-embedded Material by the Polymerase Chain Reaction," *J. Histochemistry & Cytochemistry* 39: 351-354 (1991);

(B) **Shibata** et al., "Specific Genetic Analysis of Microscopic Tissue After Selective Ultraviolet Radiation Fractionation and the Polymerase Chain Reaction," *Amer. J. Path.* 141(3): 539-543 (1992); and

(C) **McKenzie** et al. (U.S. Patent No. 5,491,062)

Background

(6) In clinical medicine, all tissue, cytology, or fluid samples removed from a patient must be evaluated by a pathologist, who is responsible for submitting a formal diagnosis upon which treatment can be based. Pathologists are required and have the legal responsibility to diagnose conditions, such as cancer, that carry major treatment implications. It is with this background that the methods and materials described in the above-referenced application, and which are generally referred to Topographic Genotyping™ (TG), were created to enable clinical specimens to be more effectively diagnosed for purposes of reaching a clinical pathology standard of care that is reliable and accurate.

(7) Prior to the methods and materials of the above-referenced application, pathology diagnosis was based entirely upon microscopic examination of cellular appearance. Microscopic examination of specimens demands chemical fixation of tissue to optimize cellular microscopic appearance for pathology review and evaluation. The rate of definitive diagnosis decreases when a specimen must be examined without the benefits conferred by chemical fixation. Also, clinical specimens cannot be subdivided for different forms of testing prior to microscopic evaluation, because of their heterogeneous nature. A pathologist will not assume responsibility for parts of specimens that are not reviewed by traditional microscopic examination. After microscopic examination, additional studies can be performed on

different portions of the submitted tissue. In fact, it is preferable that further analysis be performed on the most diagnostic sites identified by the first step of microscopy.

(8) Recently, there has been an increasing tendency to rely more heavily on preliminary diagnostic tissue biopsies or related minimal sampling techniques to reach a diagnosis of cancer (e.g., fine needle aspirations and lumpectomies). This affords the opportunity to plan specific multimodality therapy on an individual patient basis. As a result, the most important specimens tend to be small in size (biopsies). The challenge, driving the development of the methods and compositions of the above-referenced application, was to maintain information output from microscopic examination, as has been done traditionally, while nevertheless obtaining a detailed molecular analysis.

(9) In order to meet the needs for an integrated molecular pathology analysis capability (ability to do both optimal traditional microscopic examination and optimal molecular analysis), it is necessary to first allow proper chemical fixation to take place to obtain a microscopic evaluation. Using the insights derived from microscopy on an individual specimen, multiple sites bearing the greatest microscopic information regarding the condition to be diagnosed are then microdissected. Notwithstanding the small size of these microdissected samples, they are highly representative of the cellular alterations present in a given specimen. Given the need for detailed molecular analysis, these highly representative, but small-sized samples are then further subdivided into a large number of parallel molecular assays. Each molecular assay performed on the collected samples contributes valuable information towards a collective understanding of accumulated molecular changes. The small amounts of microdissected, aliquoted (divided into small equivalent portions), targeted cellular samples must undergo effective, reliable, and relatively rapid DNA amplification, in order to provide meaningful mutational damage information in the instance of cancer. From an overall perspective, it would by no means be obvious to integrate molecular analysis into standard pathology practice by aliquoting minute, fixative-treated, microdissected samples into even smaller portions for testing. Instead, one would seek to address this challenge by

securing larger sized specimen samples with which to perform the detailed mutational analysis.

(10) In the early 1990s, and to this day, it is has been shown that chemical fixation of specimens significantly reduces the amount of DNA that can be recovered by established DNA extraction protocols (J. W. Gillespie et al., "Evaluation of non-formalin tissue fixation for molecular profiling studies," 2002 *Am. J. Pathol.* 160(2): 449-57; P. Wiegand et al., "DNA degradation in formalin fixed tissues," 1996 *Pathology* 17(6): 451-4, Abstract only). Depending on the duration of chemical fixation and amount of time in paraffin block storage, recoveries of less than 10% have been demonstrated with values lower than 1% being typical of expected yield (J. W. Gillespie et al., 2002; and P. Wiegand et al., 1996). Insufficient amount of DNA in the DNA amplification reaction (*i.e.,* PCR: polymerase chain reaction) is strongly cautioned to be avoided, because artifacts and false results can ensue from the failure to provide adequate starting DNA to the reaction (J. W. Gillespie et al., 2002; Miller et al., "Assessing allelic dropout and genotype reliability using maximum likelihood," 2002 *Genetics* 160(1): 357-66; J. C. Dreesen et al., "Allelic dropout caused by allele-specific amplification failure in single-cell PCR of the cystic fibrosis delta F508 deletion," 1996 *J. Assist. Reprod. Genet.* 13(2): 112-4; G. Tully et al., "Analysis of 6 VNTR loci by 'multiplex' PCR and automated fluorescent detection," 1993 *Hum Genet.* 92(6): 554-62; L. Neihouse et al., "Allelic Dropout Confounding the Assessment of Allelic Imbalance in Small Sample Sizes," 2004 *J. Mol. Path.* 6(4): 435). This artifact is called allelic dropout and is well known to be a limiting factor in the PCR reaction. *Id.* Based on this background, the natural response to avert allelic dropout, when relying upon chemically fixed tissue as a basis for PCR amplification, would be to extract more DNA from the specimen and to take steps to guarantee that the DNA that went into the PCR reaction was of the best quality (C. De Giorgi et al., "Formalin-induced infidelity in PCR-amplified DNA fragments," 1994 *Mol. Cell. Probes* 8(6): 459-62).

Subject Matter of Patent Application

(11) In the methods of the above-referenced application, Applicants have taken the opposite approach. One aspect of difference is to keep the microdissected

sample small. By keeping the sample small, the microdissected portion is more highly representative of the lesion under examination. However, taking more tissue, the logical approach would be to mix heterogeneous areas of the specimen together in order to obtain more DNA. By doing so however, a strict relationship between microscopic findings and molecular features would be lost.

(12) In addition to reducing the yield of extractable DNA, chemical fixation also damages DNA (C. Williams et al., "A high frequency of sequence alterations is due to formalin fixation of archival specimens," 1999 *Am. J. Pathol.* 155(5): 1467-71; J. L. Bernstein et al., "Comparison of techniques for the successful detection of BRCA1 mutations in fixed paraffin-embedded tissue," 2002 *Cancer Epidemiol Biomarkers Prev.* 11(9): 809-14; C. Wong et al., "Mutations in BRCA1 from fixed, paraffin-embedded tissue can be artifacts of preservation," 1998 *Cancer Genet. Cytogenet.* 107(1): 21-7). The damage is due to a variety of mechanisms with the most important being cross linking between separate strands of DNA. *Id.* The cross linking of DNA acts to preserve the tissue constituents, but in so doing alters the physicochemical properties including solubility in organic solvents and behavior of the DNA during a PCR reaction. *Id.* Fixative cross linking is interspersed throughout the DNA and affects only a proportion of the actual DNA bases. Thus, it would have been logical to simply extract more cellular sample to accrue sufficient, good quality DNA to perform a PCR reaction. However, this approach would have been and is self-defeating, because the emphasis is placed on preserving the small size and highly representative nature of the microdissected sample in the materials and methods of the above-referenced application.

(13) Quantity and quality are closely related aspects of DNA. Therefore, the procedure in obtaining DNA should optimize both of these characteristics. Low quantity and poor quality DNA were well known to be limiting factors in the PCR reaction (J. W. Gillespie et al., 2002; C. R. Miller et al., 2002; J. C. Dreesen et al., 1996; and L. Niehouse et al., 2004). Studies have shown that methods such as phenol/chloroform extraction at the time were the preferred method for securing DNA for PCR from fixative-treated tissue (X. de Lamballerie et al., "Improved current methods for amplification of DNA from routinely processed liver tissue by PCR,"

1994 *J. Clin. Pathol.* 47(5): 466-7; K. F. Forsthoefel et al., "Optimization of DNA extraction from formalin-fixed tissue and its clinical application in Duchenne muscular dystrophy," 1992 *Am. J. Clin. Pathol.* 98(1): 98-104). Phenol-chloroform extractions were preferred, because solvent extractions are based on the physicochemical properties of the DNA, which must be maintained to be effectively extracted. It would have been logical to approach the challenge of molecular analysis of chemical fixed tissue to utilize procedures that secured the very best component of DNA, and separated good DNA from the degraded or otherwise damaged DNA. This was proven and recommended in several studies at the time (X. de Lamballerie et al., 1994; and K. F. Forsthoefel et al., 1992). Also, it would have been reasonable to use alternative extraction methods that are based on DNA maintaining a particular level of quantity and physicochemical integrity (A. M. de Roda Husman, et al., "Processing of long-stored archival cervical smears for human papillomavirus detection by the polymerase chain reaction," 1995 *Br. J. Cancer.* 72(2): 412-7; and X. de Lamballerie et al., 1994).

(14) Again, an opposite tactic was chosen for the materials and methods of the above-referenced application, because there was an absolute requirement to enable the small, representative microdissected sample to prove sufficient for PCR and during further steps in the molecular analysis process. Contrary to conventional wisdom, sample handling as a crude lysate was chosen as it gave all available DNA the opportunity to contribute to the PCR reaction. Recommendations against a crude lysate were documented in the literature (*see, e.g.,* P. J. Coates et al., "Simplified procedures for applying the polymerase chain reaction to routinely fixed paraffin wax sections," 1991 *J. Clin. Pathol.* 44(2): 115-8; K. F. Forsthoefel et al., 1992; B. B. Rogers et al., "Analysis of DNA in fresh and fixed tissue by the polymerase chain reaction," 1990 *Am. J. Pathol.* 136(3): 541-8; S. E. Goelz et al., "Purification of DNA from formaldehyde fixed and paraffin embedded human tissue," 1985 *Biochem. Biophys. Res. Comm.* 130(1): 118-26). Therefore, at the time a crude lysate would not have been an intuitively obvious way to proceed.

(15) One means to obtain more DNA from a given fixative-treated specimen is to histologically cut the tissue section at greater thickness (*see* cited referenced by

J. Ben-Ezra et al., "Effect of fixation on the amplification of nucleic acids from paraffin-embedded material by the polymerase chain reaction." 1991 *J. Histochem. Cytochem.* 39(3): 351-4). Standard tissue sections are cut at 4-5 microns thickness. Increasing the tissue section thickness provides correspondingly elevated amounts of sample for DNA extraction. This simple, logical approach was apparently taken as a means to enhance molecular analysis (*Id.*).

(16) The strategy for developing materials and methods of the above-referenced application was counterintuitive, because the use of standard 4-5 micron thick sections was preferred and not using thicker sections. The reason for this was to keep tissue section preparation simple and as close to routine practice as possible so that routine histology laboratories would not find it burdensome. Histotechnologists must work quickly given their large case load. Constantly adjusting microtome settings would not be a well received change of practice. Thicker tissue sections have a tendency to curl or break apart and are much more difficult to manage, often falling off the glass slide to which they are intended to firmly adhere. Most importantly, pathology governing bodies caution against the unnecessary sacrifice of precious tissue specimens, which occurs must faster when tissue sections are cut thick (S. R. Florell et al., "Preservation of RNA for functional genomic studies: a multidisciplinary tumor bank protocol," 2001 *Mod. Pathol.* 14(2): 116-28; and L. G. Dressler et al., "Policy guidelines for the utilization of formalin-fixed, paraffin-embedded tissue sections: the UNC SPORE experience," 1999 *Breast Cancer Res Treat.* 58(1): 31-9). The methods and compositions described in the above-referenced application respects need for careful use of tissue by not requiring thicker sections. That is not to say that thicker or thinner sections cannot be used if provided. Rather, the methods and compositions described in the above-referenced application do not require unusual requirements on the part of the histopathology laboratory, thereby maintaining a standard operating procedure.

(17) Thus, the materials and methods of the above-referenced application use a small quantity, crude lysate. The lysate is composed of minimal amounts of fixative damaged DNA and is further subdivided into a number of samples upon which parallel molecular analysis can be performed. The process claimed in the

instant application solved numerous problems and which went against standard operating procedure and scientific practice at the time in a variety of ways. There were no recommendations at the time on how to handle a crude DNA-containing lysate for a PCR reaction. Nor was there any confidence that the methods described in the above-referenced application would work effectively.

(18) The crude lysate obtained using the described methods is a suspension of tissue fragments varying greatly in size at the molecular level; large tissue chunks with degraded fragments of strands of DNA with the full range of intermediate forms coexist in the crude lysate. Practitioners have cautioned against having undigested tissue fragments in the interfering with PCR amplifiability (Z. P. Ren et al., "Recovering DNA and optimizing PCR conditions from microdissected formalin-fixed and paraffin-embedded materials," 2000 *Pathobiology* 68(4-5): 215-7). In fact, there is scientific literature at the time alluding to interfering factors inhibiting PCR (S. Kosel et al., " Use of neuropathological tissue for molecular genetic studies: parameters affecting DNA extraction and polymerase chain reaction," 1994 *Acta Neuropathol (Berl).* 88(1): 19-25; and S. F. An et al., "Removal of inhibitor(s) of the polymerase chain reaction from formalin fixed, paraffin wax embedded tissues," 1991 *J. Clin. Pathol.* 44(11): 924-7). The nature of the interfering factors was not known, but one manner to address this was to increase tissue digestion and/or utilize a DNA extraction method such as phenol/chloroform (S. Kosel et al., 1994 and S. F. An et al., 1991). Novel reasoning was used here to utilize novel attributes of sample centrifugation which would create a solubility gradient based on tissue fragment size, composition and behavior during centrifugation. However, no scientific articles at the time mentioned the use of centrifugation in relation to crude lysate management in this context. It was hypothesized that centrifugation would pellet the large interfering fragments thereby removing this "interfering factor". There is no literature at the time that suggested that centrifugation would in fact remove an "interfering factor. Centrifugation would also cause the highly degraded, small strands of damaged DNA to move to the surface and/or to be diluted in the fluid phase of the centrifuged crude lysate. Thus, use of a centrifugation step to enhance the performance of a crude lysate was not obvious or commented upon in the literature, nor would there

be an expectation that it would work in a PCR reaction. Aliquots for individual PCR reactions would then be taken from just above the pellet, where the DNA was neither in large fragments nor in a highly degraded form. This innovative procedure optimized the crude lysate in the state that it existed and unexpectedly led to effective PCR. Thus, the centrifugation step was not an obvious solution to managing the type of crude lysate that exists in this context for preparing a lysate for use in PCR.

(19) Having proven the hypothesis, it then was possible to make further innovations that proved highly complementary to the overall objective of rendering minute, fixative-treated microdissected samples suitable for robust PCR amplification. In fact, it was determined that it was important not to overload the sample, because that results in too much interfering large fragments of tissue. Similarly, it was important not to cut the tissue sections too thick, because that too only increased the likelihood of large fragments existing in the crude lysate. Tedious, low efficiency extraction procedures such as phenol/chloroform were in fact discouraged now that it was evident that sufficient good quality, amplifiable DNA was in fact present in the crude lysate. Traditional DNA extraction procedures would lead to loss of specimen and DNA, both good and poor forms, leading to certain failure of the procedure. Even stained tissue sections and stained cytology preparations could be made to work effectively by manipulating the crude lysate DNA content that was ideal for PCR amplifiability (J. M. Rae et al., "Genotyping for polymorphic drug metabolizing enzymes from paraffin-embedded and immunohistochemically stained tumor samples," 2003 *Pharmacogenetics.* 13(8): 501-7; T. Murase et al., "Influence of histochemical and immunohistochemical stains on polymerase chain reaction," 2000 *Mod. Pathol.* 13(2): 147-51; and M. P. Burton et al., "Comparison of histologic stains for use in PCR analysis of microdissected, paraffin-embedded tissues," 1998 *Biotechniques* 24(1): 86-92). However, staining of tissue sections causes dye binding to DNA and was recommended to be avoided in the literature (T. Murase et al., 2000; and M. P. Burton et al., 1998). With the materials and methods taught in the above-referenced application, even stained tissue and cells can be used to obtain excellent correlative molecular pathology information.

(A) Ben-Ezra Reference

(20) The Ben-Ezra reference is directed to amplification of DNA from a large specimen. The specimen is 6 µm thick; the area of the specimen is not stated. However, an average size of a fibroadenoma is approximately 2 cm in diameter. Therefore, a specimen that is 6 µm thick and 2 cm in diameter can be assumed to have approximately 2 million cells.

(21) On page 352 of Ben-Ezra, the authors state "[a] single 6 µm [thick] section was cut and placed in a 1.5 mL polypropylene tube." From that statement, I assume that the authors removed a 6 µm thick slice of the entire tissue block and surrounding paraffin support and placed that entire tissue block slice in the 1. 5 mL tube. From that assumption of standard pathology practice and the average size of the fibroadenoma, I arrived at the calculation of about 2 million cells being present in the sample. This is an extremely large sample, which by the described methods of the instant application would result in overloading the sample for PCR. The claimed methods use on average 1 mm in diameter microdissected targets and generally 6 µm thick or less (typically 4 µm) sections. This is about 4000X less tissue than recommended by Ben-Ezra.

(22) Ben-Ezra performs a boiling step, which yields DNA. After deparaffinization, the tissue containing the DNA was placed in 50 µL of water. The DNA was extracted from the tissue by boiling the 50 µL-sample for 10 minutes. *See* page 352.

(23) Ben-Ezra used 45 µL of the 50 µL lysate (90% of the sample) in the PCR reaction. Thus, the PCR reaction was performed on the DNA of approximately 1.8 million cells, or nearly the whole sample for just one PCR reaction, which is highly wasteful and impractical for running a plurality of marker assays. The methods described in the subject application use only about 1 µL of the 50 µL sample, or 2% of the sample. This is relevant, because a mutational profile of about 15 to about 20 or more separate PCR reactions can be run in parallel as needed from each microdissected sample. Thus, the amount of the sample used is approximately 50X more efficient than what is taught by Ben-Ezra, and when

combined with the number of cells Ben-Ezra required in his reaction, the efficiency is even greater.

(B) Shibata Reference

(24) Shibata uses very different approach from the described methods and materials and from what is described by Ben-Ezra. The Shibata method is called Selective Ultraviolet Radiation Fractionation (SURF). Standard 4-micron thick tissue sections are placed on acetate sheets instead of glass slides. Additionally, the tissue slices require heating to 110°C for 5 minutes to bond the tissue to the slide. *See* Shibata, p. 540. Unaltered DNA (e.g., DNA that is not obtained from fixative treated and/or stained cells) is quite resistant to boiling, and thus boiling unaltered DNA is acceptable for purposes of its purification. Chemically modified DNA, such as that obtained from stained and/or fixative treated DNA, is less stable when heated. Thus, the heating step would further detrimentally impact the DNA quality of DNA, which is already present in small amounts given the small sample size.

(25) Another negative aspect of the SURF method is that tissue sections are described as not sticking well and wrinkling while placing them on the acetate sheets. This would not be tolerated generally by histologists, who do not have the luxury to spend a great deal of time on such complex procedures. Then a felt-tipped pen is used to place an ink dot "precisely" on the microscopic area of interest. Dried tissue is like a sponge and the natural osmotic action of the tissue with the felt-tipped pen will result in the tissue soaking up the ink in an uncontrolled fashion. The ink pen is then thrown away after the single use, which is highly wasteful. *See* Shibata, p. 540. The tissue is now exposed to ultraviolet ("UV") light with the expectation of selective degradation of unwanted DNA, which is not protected by the ink dot. The procedure is not practical and does not seem to have been reported in the literature by any other pathologist except the authors.

(26) As stated in ¶19 above, staining tissue is detrimental to DNA for amplification purposes by polymerase chain reaction (PCR). Formalin fixation also damages the DNA for purposes of amplification. *See* ¶10-11 and 19 above. Finally, in Shibata, the tissue was not only formalin-fixed and stained, but also dotted with

ink, which can also detrimentally impact the quality of the DNA for amplification purposes by PCR. Finally, the ink may not fully protect the DNA of the inked-tissue from the UV treatment, thus reducing the quality and quantity of the DNA for processing by the claimed methodology.

(27) Shibata includes a great deal of tissue that presumably has been degraded by ultraviolet light, but which I believe more likely is contributing to the PCR reaction.

(28) In Shibata, human papilloma virus (HPV) DNA is targeted for amplification. Viral DNA is present in very large amounts in infected cells.

(29) Shibata also reports on analysis of a single copy P53 gene. Shibata uses very powerful detection techniques: P^{32} labeling and dot blots for PCR and 30 minute UV transillumination to see the PCR products from the papilloma virus. *See* Shibata, p. 540. The authors also used 42 cycles of PCR amplification. They are making very little product and hoping to see it by pushing the detection system. Thus, for single copy gene analysis, the methods taught are distinguishable from the claimed methods. Additionally, the long-term exposure of the amplied product to X-ray film is unacceptable under a standard of care for clinical pathology that may have to provide a definitive diagnosis for the patient within a few days, and not several weeks.

(30) Most importantly, just like Ben-Ezra, Shibata teaches a highly inefficient method and does not suggest the steps of the claimed methods. Additionally, the teachings of Shibata and Ben-Ezra provide no motivation to combine their methods. The methods cannot work together. Alone, each reference in its own manner would have led the user at the time to adopt techniques that operate in an environment of extremely ineffective PCR. Ben-Ezra does this by massive use of tissue. Shibata does this by pushing to the limit the detection systems and suggesting a methodology, which has been incapable of being replicated by anyone other than the authors of the reference without great difficulty. The Shibata technique therefore would not be feasible for consistent use in pathology laboratories.

(C) McKenzie et al. Patent

(31) The methodology of McKenzie applies to fresh specimens. Nothing in the patent helps the reader to understand the challenges inherent in dealing with fixative treated specimens, let alone stained and/or ink dotted specimens. As with the articles cites above (Ben-Ezra and Shibata), a closer reading of the methodology shows it to be highly inefficient. There would not have been any suggestion to use the procedure of McKenzie for fixative treated cells or tissue. In McKenzie, the reader is instructed to use one million fresh cells. *See* col. 9, lines 12-13 and col. 5, lines 51-65. Note that on line 65 it directs the reader to Section I.D.2.c., which is the boiling step of more than 1 million cultured fresh cells. The number of cells utilized is about 3 orders of magnitude higher than that typically encountered in microdissection based analysis of surgical pathology or cytology specimens in clinical practice. It must also be noted that detection of a mycoplasma in infected cells is detection of DNA, which is present many thousand fold higher than host cell DNA. So, the technique requires far greater numbers of target templates of DNA than that seen when using the materials and methods of the above-referenced application. Additionally, the application does not suggest how to work with cells other than fresh cells, and the described methods of McKenzie would not be effective with fixative and/or stained cells. Also the technique requires purified DNA to the point where it can be accurately measured so that it can be adjusted as needed. *See* column 9, line 23. Determination of DNA concentration is not possible in fixative treated tissues, because the claimed methods are not sufficiently pure to allow DNA concentration determination.

(32) Additionally, McKenzie describes a method of DNA genotyping of mycoplasma tRNA genes in cells. Mycoplasma tRNA is present in many copies per cell.

(33) McKenzie also teaches the use of two-phenol/chloroform steps on $5x10^5$ to $2x10^6$ fresh cells. *See* col. 8, lines 60-61.

(34) Simply put, McKenzie does not suggest using its described materials and methods with fixative treated specimens. McKenzie cannot be combined with the other articles, because shortcomings of each are not addressed by the other

references. The key aspect is the reality of very small quantities of fixative treated DNA, which is not properly addressed by any of the articles.

(D) References Combined

(35) I understand that the Office Action cites Ben-Ezra as it primary reference. I further understand that the references of the McKenzie patent and the Shibata reference have been combined to form an obviousness rejection. The teachings of these references alone do not suggest the claimed invention for the reasons set forth above.

(36) A pathologist in 1994 doing genetic analysis of a tissue sample would have extracted the DNA and would have performed a phenol/chloroform precipitation step, which was standard practice at that time as discussed above. It would not have been expected at the time that a PCR amplification of DNA by the described method that had not undergone a phenol/chloroform precipitation step would have produced successful and reliable results.

(37) It was unexpected in 1994 that such a small sample of cells (a microscopic sized target from any tissue, but especially fixative-treated and/or stained tissue) would successfully and reproducibly produce genetic data. Moreover, it is even further unexpected that the genetic analysis can be reproducibly and successfully performed on one-fiftieth of the extracted sample.

CONCLUSION

(38) I further declare that all statements made herein of my own knowledge are true and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issuing thereon.

Date: July 19/05

Sydney D. Finkelstein